AN INTEGRATED SELEX MICROFLUIDIC SYSTEM FOR RAPID SCREENING OF INFLUENZA VIRUS-SPECIFIC APTAMERS

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ABSTRACT

An integrated microfluidic system was developed to automatically perform systematic evolution of ligands and exponential enrichment (SELEX) process using combinational single-stranded DNA (ssDNA) library for rapid screening of highly specific aptamers for influenza viruses in this study. This integrated microfluidic system consisted of several modules on a single chip. With this system, the specific aptamers were screened successfully with high affinity for influenza viruses after several repeated extraction and amplification runs. Therefore, it may provide a useful platform for specific aptamers screening, which can be a promising biomarker to recognize viruses for fast diagnosis.

KEYWORDS: Influenza virus, aptamer, SELEX, microfluidics

INTRODUCTION

Influenza viruses are a group of RNA viruses belonging to Orthomyxoviridae family. They are classified into three types (A, B and C) according to different surface antigens. Some strains of influenza viruses are notorious for high morbidity and mortality, which causes severe global pandemic diseases, resulting in 250,000 and 500,000 deaths each year [1]. Furthermore, the number of deaths caused by influenza viruses can be up to millions in particular pandemic years. Early and rapid diagnosis of influenza viruses is therefore crucial for influenza pandemic control and prevention. Traditional methods for detection of influenza viruses include virus culture, enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition test, reverse-transcription polymerase chain reaction (RT-PCR), and rapid influenza diagnostic tests. However, some drawbacks have been reported for these methods. For instance, ELISA requires a lengthy process, exhibits relatively low sensitivity, low specificity and consumes relatively high reagents. Most importantly, anti-influenza antibodies are sensitive to humidity and temperature and therefore require delicate apparatus for storage. It is therefore crucial to screen a reliable biomarker to recognize viruses. In this study, we reported a microfluidic platform to screen aptamers for recognizing influenza viruses. To the best of our knowledge, the diagnosis of influenza A virus using aptamers is the first attempt in literature.

Aptamer is single-stranded oligonucleotides that can be synthesized by artificial methods and selected for targets of interest in vitro [2]. It was demonstrated that the aptamer has high specificity and affinity to bind with various kinds of targets, including small molecules, proteins, drugs, viruses, and even cells from a combinatorial ssDNA library. Compared with the antibody, the aptamer has higher affinity, higher specificity, rapid and cheaper synthesis and requires non-immune responses. The processes of screening specific aptamers is called systematic evolution of ligands and exponential enrichment (SELEX) technology that performs iterative rounds of isolation and amplification processes of ssDNA [3]. At each screening round, the individual specific oligonucleotides could bind to targets with high affinity and those unbound oligonucleotides are filtered out. However, the SELEX is an iterative process that requires a large amount of samples and reagents to perform DNA extraction and polymerase chain reaction (PCR) amplification. Moreover, the traditional SELEX process is usually time-consuming, labor-intensive, and requires expensive large-scale equipment.

In this study, a new automatic platform was developed to perform the entire SELEX process for rapid screening of specific aptamers for influenza viruses. This virus-SELEX system is smaller in size, consumes less samples and reagents, and faster in the SELEX process when compared with the traditional SELEX method. Specific aptamers for influenza A/B viruses were successfully screened by using the developed system.

DESIGN AND FABRICATION

The virus-SELEX process was performed on an integrated microfluidic system for screening specific aptamers of influenza viruses. The positive and negative selections were alternately processed to obtain highly specific aptamers with high affinity for influenza viruses. The operating process of screening aptamers specifically for influenza A virus was schematically shown in Fig. 1. Briefly, the ssDNA library was incubated with influenza A virus and magnetic beads coated with anti-influenza A antibody. The magnetic beads could capture ssDNA specific to influenza A virus, which was defined as positive selection. Then, the captured ssDNA was amplified by PCR. To obtain apatmers that can bind influenza A virus but not influenza B virus, negative selection using magnetic beads coated with anti-influenza B antibody was performed after positive selection. After the incubation process, the supernatant of reactant were collected as a test sample for the next round. The positive and negative selections were alternately performed for 4~5 rounds to obtain aptamers for influenza viruses with higher specificity. Similarly, in order to screen specific aptamers for influenza B, the influenza A virus was used in negative selection. By performing iterative process of screening and amplification, the aptamers specific to influenza viruses can be screened successfully.

The schematic illustrations of the virus-SELEX chip in cross-sectional view and top view were shown in Figs. 2 and 3, respectively. This chip was made of two polydimethylsiloxane (PDMS) layers and one glass plate. In addition, it contained several modules, including suction-type, pneumatic micropumps, micromixers, reagent and sample chambers, a PCR module and an aptamer extraction module were integrated on a single chip for automating the entire screening process. Figure 4 shows a photogragh of the virus-SELEX chip. The dimensions of the chip were measured to be 5.0 cm in width and 5.5 cm in length. The extraction and amplification of specific influenza virus-aptamers were examined by gel electrophoresis.

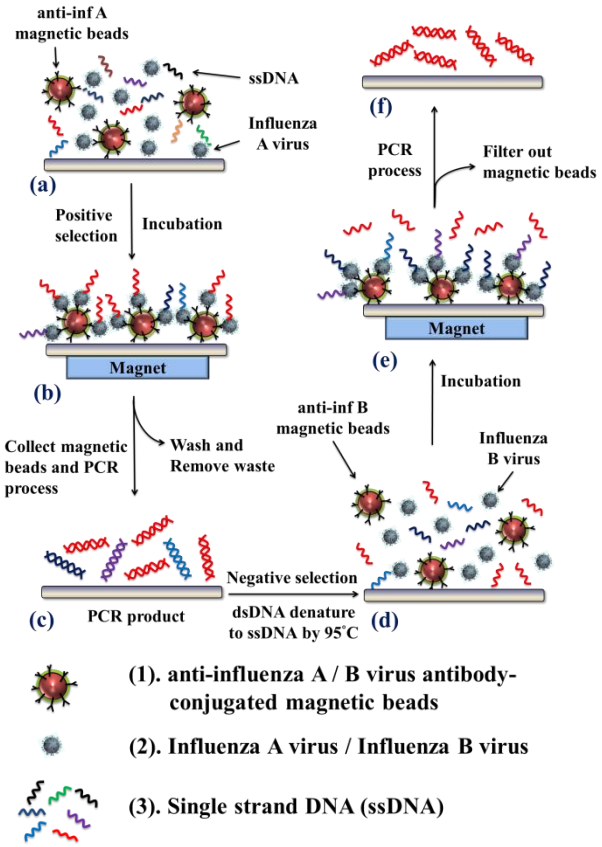

Figure 1: The SELEX processes of screening aptamers specific to influenza viruses, including incubation, selection and amplification were performed. (a) The ssDNA library was incubated with influenza A virus and anti-inf A magnetic beads for positive selection. (b) Non-bound DNA sequences were washed away. (c) The bound DNA sequences were collected by magnetic beads and amplified by PCR. (d-f) The PCR products were incubated with the influenza B virus to filter out the sequences that can bind to influenza A virus and influenza B virus both, and then the specific ssDNA to the influenza viruses were amplified by PCR.

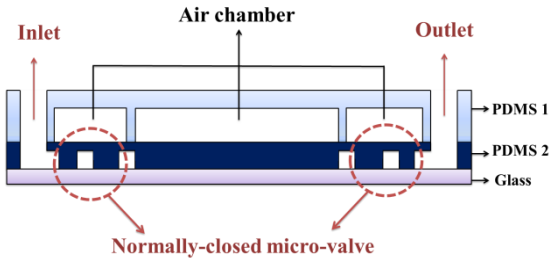

Figure 2: The cross-section view of the SELEX chip for screening of aptamers specific for influenza viruses.

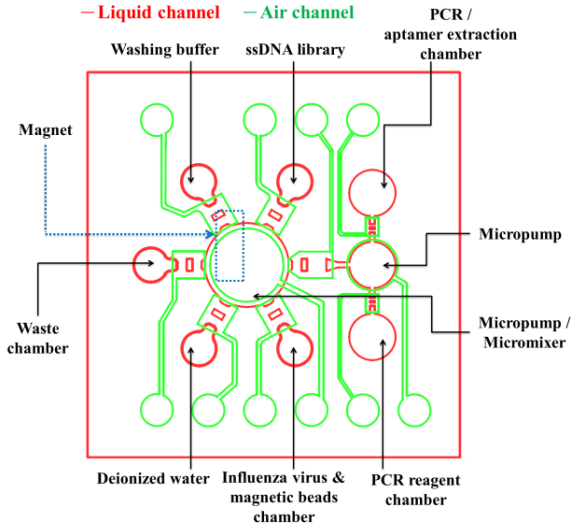

Figure 3: The schematic illustration of the SELEX chip.

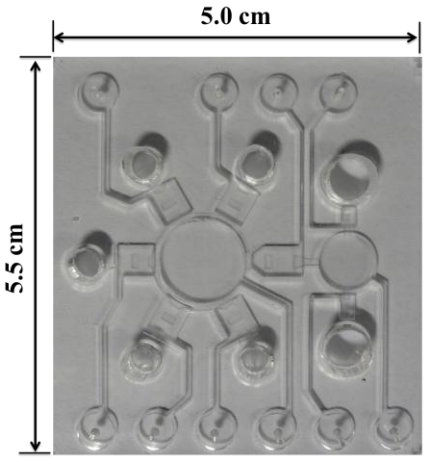

Figure 4: A photograph of the virus-SELEX chip. The dimensions of the chip were measured to be 5.0cm × 5.5cm.

RESULTS AND DISCUSSION

The microfluidic components were characterized first. For instance, in this study, an active micromixer was developed to perform the incubation process for ssDNA library and magnetic beads. The normalized concentration profile across the mixing chamber is shown in Fig. 5, indicating that the micro-mixer can be used to mix the ssDNA and magnetic beads successfully within 2 seconds. (Note that D^+ is the normalized location across the mixing chamber and C^+ is the normalized concentration.)

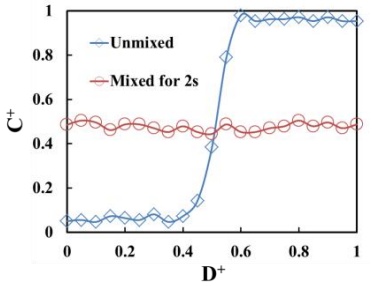

Figure 5: The normalized concentration profile across the mixing chamber.

The 72 bp of amplified specific aptamers for influenza viruses were examined by gel electrophoresis after each round of the SELEX process. Figure 6 shows the gel electrophoresis results for the PCR products after each round in the SELEX process for positive selection. It indicates that 72 bp of the ssDNA can be successfully screened. In addition, these specific aptamers were further incubated with other type of influenza virus/magnetic beads as the negative selection. The gel electrophoresis results of the specific aptamers performed by negative selection were shown in Fig. 7. These results showed that the microfluidic system can be used for screening a specific aptamers efficiently without manual operation.

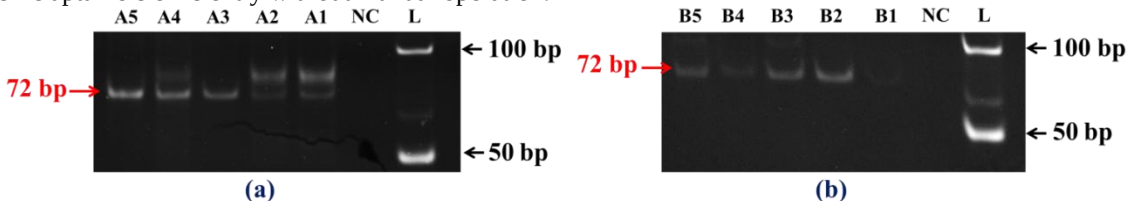

Figure 6: Gel electrophoresis showed that 72 bp of amplified products after various rounds by SELEX positive selection for influenza viruses can be successfully screened. (a) The PCR products of influenza A virus after 1, 2, 3, 4 and 5 rounds by SELEX, respectively. (b) The PCR product of influenza B virus B after 1, 2, 3, 4 and 5 rounds by SELEX, respectively. L is 50-bp DNA ladders.

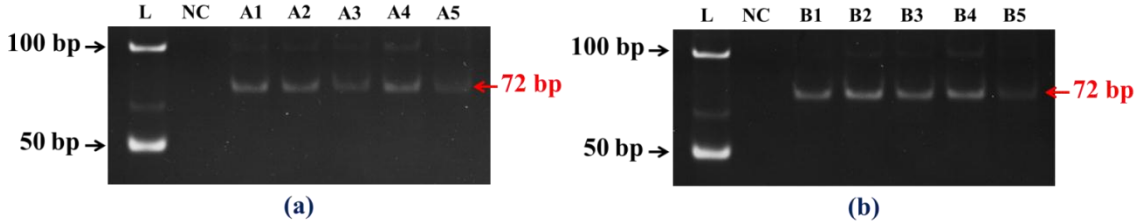

Figure 7: Gel electrophoresis showed the PCR products after several rounds of negative selection from ssDNA library amplification. (a) The amplification of influenza A virus after 1, 2, 3, 4 and 5 rounds by SELEX, respectively. (b) The amplification of influenza B virus after 1, 2, 3, 4 and 5 rounds by SELEX, respectively. L is 50-bp DNA ladders.

CONCLUSION

In this study, a new integrated microfluidic system was developed for rapid screening of aptamers specific to influenza viruses by using the virus-SELEX technology. The specific aptamers can be successfully isolated and efficiently enriched from the random ssDNA library. Compared to the traditional SELEX method, this integrated virus-SELEX microfluidic system has the advantages of automation, rapid screening, fewer consumption in samples and reagents. The screened aptamers can be further used for diagnosis of influenza A/B viruses.

ACKNOWLEDGEMENTS

The authors would like to thank the National Science Council in Taiwan for financial support (NSC 101-2120-M-007-014).

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